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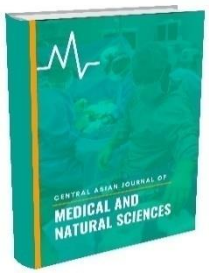

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Bacteriologic and Immunologic Study for Some Patients with Psychological Stress

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Abstract: In recent decades, many stresses have increased on members of society, which has led to the emergence of many psychological diseases related to psychological stress. Therefore, this study aims to find the relationship between individuals who suffer from psychological stress and increased number of intestinal bacteria, as well as to study some immunological parameters related with stress. The study enrolled 180 participants including 97 (53.9%) patients suffering from schizophrenia disease and 53 (29.4%) patients with Alzheimer's disease, as well as 30 (16.7%) persons as control group. Blood samples and clinical feces were collected from patients in Al-Rashad and Ibn Al-Rushd Hospitals in Baghdad province during the period August 2022 to March 2023, and the information of each sample was taken in its own questionnaire. The bacteria were diagnosed by phenotypic, microscopic, and biochemical tests. the results revealed that *Klebsiella spp*. was the most bacterial species in schizophrenia patients which reached to 30 (48.4%), while the number of *Escherichia coli* isolates was 12 (19.3%). In Alzheimer's patients, *Klebsiella spp*. was the most common bacterial species in schizophrenia patients which reached to 11 (35.5%), while the number of *Escherichia coli* isolates was 7 (22.6%). The study include estimation the levels of immunological parameters IL-6 and TNF-α using ELISA. The results revealed elevation the level of IL-6 ($P<0.05$) in sera of patients in comparison with healthy control group , which reached to (2.73±0.68) and (3.06±0.83 pg/ml) for patients with schizophrenia and Alzheimer's diseases respectively , while it was 0.94±0.16 Pg/ml in healthy control group. In addition, the level of tumor necrosis factor- alpha (TNF-α) showed a significant increase ($P<0.05$) in patients with schizophrenia (5.18±1.25 pg ml) and Alzheimer's disease (4.91±0.77 p/ ml) compared

to the healthy control group (2.16±0.37 pg/ml). In conclusions, the psychological diseases (schizophrenia, Alzheimer's) associated with stress increased the number of intestinal bacteria in feces samples of patients in comparison with healthy control group, also increased the levels of the cytokines IL-6 and TNF- α in patients compared with healthy control group.

Key words: Alzheimer's disease, schizophrenia, IL-6, TNF- α

Introduction

The concept of stress is known as the body's general reaction to changes that occur in the environment [1], and many studies have indicated that psychological stress has many effects on immune functions [2-3]. Stress reactions lead to a response from the neuroendocrine system, which includes the hypothalamic-pituitary-adrenal axis. This leads to stimulation of the sympathetic nervous system to secrete glucocorticoids and mineralocorticoids (catecholamine). This response is followed by an increase in oxidative stress [4]. Many studies have confirmed that psychological stress is linked to changes in physiological functioning, including immune changes. High levels of psychological stress have been associated with decreased control of latent herpes virus infections, humoral immune responses to immunization, and greater susceptibility to immunization. Infectious diseases and poor wound healing [5-6]. The link between stress and immune functions has received great attention from researchers in the past decades [7-8], as dysregulation of neuroendocrine and immune system functions resulting from chronic stress has been linked to psychological and physiological disorders. psychological and physiological disorders, including depression, atherosclerosis, asthma, cardiovascular disease, cancers, and the development of AIDS [9-10]. Some studies have reported higher levels of Actinobacteria and Eggerthella in depression, and Enterobacterales and Enterobacteriaceae in anxiety, including Klebsiella, Escherichia coli, Salmonella, Serratia, and Proteus [11]. In another study, a higher abundance of Eggerthella, bacteria belonging to the Enterobacteriaceae family, and a lower abundance of Faecalibacterium and Coprococcus were commonly observed in adults with anxiety, depression, bipolar disorder, psychosis, and schizophrenia [12]. In recent decades, many life pressures on members of society have increased, leading to the emergence of many psychological diseases related to psychological stress. Therefore, this study aims to find the relationship between individuals who suffer from psychological stress and infection with some bacterial infections, as well as studying some immune parameters affected by stress. Through the following:

- Isolating and diagnosing some types of bacteria from individuals suffering from some mental illnesses.
- Studying the concentrations of some cytokines in the sera of patients in different age groups.

Materials and Methods

Specimens Collection

Blood and clinical discharge samples were collected for patients with schizophrenia and Alzheimer's from Al-Rashad Hospital and Ibn Al-Rushd Hospital in the city of Baghdad for the period from August 2022 to March 2023, and information was taken through their questionnaire form.

Bacterial Identification

Bacteremia was diagnosed based on the following aspects:

Microscopic examination

Bacterial colonies were identified based on the morphological characteristics of the germ cells under the microscope and through the nature of their interaction with a cloud stain, which shows the type of interaction and the shape and arrangement of the germ cells.

Biochemical Tests

The following biochemical tests were performed.

- Oxidase Test
- Catalase Test
- Indol Test
- Urease Test
- Methyl red Test
- Voges – Proskauer (VP) Test
- Citrate Utilization Test
- Motility Test
- Mannitol Fermentation Test

Determination the level of Interleukin-6

Human IL-6 was quantified by enzyme-linked immunosorbent assay (ELISA) in serum using the IBL International GmbH Kit Flughafenstr. 52A, D-22335 Hamburg, Germany (Mindray 96-A microplate reader, Shenzhen 2007-2010).

Determination the level of serum TNF-α

An enzyme-linked immunosorbent assay kit has been used to estimate TNF-alpha concentrations in serum and plasma.

Statistical analysis

The results of the current study were analyzed statistically using the statistical analysis program called Minitab, as well as using the Excel program, and the results were presented in the form of arithmetic mean ± standard error. The results of the study were analyzed statistically according to the ANOVA test for the purpose of detecting significant differences by comparing the arithmetic means of the experimental groups. different using Duncan's multiple test [13].

Results and discussion

Samples distribution

The current study included collecting 180 samples, including 30 (16.7%) control group, 97 (53.9%) schizophrenia group, and 53 (29.4%) Alzheimer's group (Table 1). The results found that 102 (56.7%) of the total samples showed positive results for bacterial growth on optimal media, including blood agar and MacConkey agar. On the other hand, 78 (43.3%) of the total samples showed a negative result for bacterial growth.

Table (1): Distribution of study samples according to bacterial growth

Disease type	Positive growth	Negative growth	Total
Control	9(٪30.0)	21(٪70.0)	30(16.7%)
Schizophrenia	62(61.5٪)	35(38.5٪)	97(53.9%)
Alzheimer's	31(60.3٪)	22(39.7٪)	53(29.4%)
Total	102(56.7٪)	78(43.3٪)	180(100%)

Identification

The shape, diameter and shapes of bacterial colonies were determined on blood agar, MacConkey agar. As well as microscopic and biochemical examinations, which included qualitative tests for each species. Table (2) also shows the numbers and percentages of bacterial samples that were isolated from patients with schizophrenia and Alzheimer's in the form of single isolates as well as double isolates. The table shows that 72.5% of the bacterial isolates were single for only one type of bacteria, while 27.5% of the total 102 isolates were Mixed isolates, where more than one type of bacteria was isolated in one sample. While Table (3) shows the numbers and percentages of each type of Gram-negative bacteria that were isolated.

Table (2): Numbers and percentages of bacterial samples among study patients

Isolate types	Number	Percentage
Single	74	72.5%
Mixed	28	27.5%
Total	102	100

Table 3: Numbers and percentages of Gram-negative bacteria samples

Bactria types	Number	Percentage
Klebsiella spp.	42	41.2%
Escherichia coli	21	20.6%
Pseudomonas aeruginosa	14	13.7%
Salmonella spp.	10	9.8%
Proteus mirabilis	7	6.9%
Serratia spp.	4	3.9%
Enterobacter cloacae	4	3.9%
Total	102	100

The different types of bacteria in schizophrenia patients are also shown in Table (4), as the results showed that Klebsiella spp. It was the most isolated among the different types of bacteria, reaching 30 (48.4%), while the number of Escherichia coli isolates reached 12 (19.3%), while Pseudomonas aeruginosa and Salmonella spp. The number of isolates was 6 (9.7%) and 6 (9.7%), respectively. On the other hand, the number of isolates of Proteus mirabilis, Serratia spp. and Enterobacter cloacae are 4(6.5%), 2(3.2%), and 2(3.2%), respectively.

Table (4): Numbers and percentages of bacterial samples in schizophrenia patients

Bactria types	Number	Percentage
Klebsiella spp.	30	48.4%
Escherichia coli	12	19.3%
Pseudomonas aeruginosa	6	9.7%
Salmonella spp.	6	9.7%
Proteus mirabilis	4	6.5%

Serratia spp.	2	3.2%
Enterobacter cloacae	2	3.2%
Total	62	100

On the other hand, Table (5) shows the different types of bacteria in Alzheimer's patients, where the results showed that Klebsiella spp was the most isolated among the different types of bacteria, reaching 11 (35.5%), while the number of Escherichia coli bacteria isolated reached 7 (22.6%), while Pseudomonas aeruginosa and Salmonella spp. The number of isolates was 5 (16.1%) and 3 (9.7%), respectively. On the other hand, the number of isolates of Proteus mirabilis, Serratia spp. and Enterobacter cloacae are 2(6.4%), 1(3.3%), and 2(6.4%), respectively.

Table (5): Number and percentage of bacteria samples in Alzheimer's patients

Bactria types	Number	Percentage
Klebsiella spp.	11	35.5%
Escherichia coli	7	22.6%
Pseudomonas aeruginosa	5	16.1%
Salmonella spp.	3	9.7%
Proteus mirabilis	2	6.4%
Serratia spp.	1	3.3%
Enterobacter cloacae	2	6.4%
Total	31	100

In the past few years, scientists have begun to unravel the evolution and symbiosis of humans and microbes. Microbes include viral, bacterial, fungal and archaeal organisms. The microbiome is the entire microbial genomic code. The microbial proteome, specifically, is microbial genes that are actually expressed and translated into proteins [14]. In recent years, variation in gut bacteria between schizophrenia patients has been compared with healthy controls. In contrast to the healthy intestine, facultative anaerobes such as Cronobacter spp. and Enterococcus spp. It was found in the intestines of ninety drug-free schizophrenia patients [15]. In another study, the results show an increase in some pathogenic bacteria in schizophrenia patients. In particular, there was an increase in the numbers of Clostridium and Klebsiella bacteria [16]. Another study based on metagenomic analysis of the normal gut microbiome indicated an increased level of Klebsiella in the early stages of disease diagnosis compared to healthy people [17], and these studies confirm and agree with the results of the current study in terms of an increase in Klebsiella bacteria in patients with schizophrenia and Alzheimer's disease.

Immunological examinations

The results of the current study showed significant changes ($P<0.05$) in the levels of interleukin-6 in both schizophrenia and Alzheimer's patients compared to the control group. Interleukin-6 levels showed a significant increase in both schizophrenia (2.73± 0.68) and Alzheimer's (3.06± 0.83) patients compared to the control group (0.94±0.16), as in Table (6). In addition, tumor necrosis factor alpha (TNFα) levels showed a significant increase in both schizophrenia (5.18± 1.25) and Alzheimer's (4.91± 0.77) patients compared to the control group (0.37±2.16).

Table (6): Interleukin-6 and TNFα levels in the study groups

Disease type	IL-6 (pg/ml)	TNFα (pg/ml)
Control	0.94±0.16 b	2.16± 0.37 b
Schizophrenia	2.73± 0.68 a	5.18± 1.25 a
Alzheimer's	3.06± 0.83 a	4.91± 0.77 a

The results of the current study also showed increased levels of IL-6 and TNF-alpha in samples of schizophrenia patients. The elevation of these cytokines is consistent with the pro-inflammatory state well described in schizophrenia and reported by some studies [18-19]. Increased IL-6 and TNF-alpha are both proinflammatory mediators produced predominantly by macrophages. The results of the current study agreed with a group of previous studies that indicated increased levels of IL-6 associated with schizophrenia [18-20]. In a study conducted by Naudin et al., [21], higher levels of TNF-a were found in patients with schizophrenia with no relationship between global, negative, and positive subscale scores. However, in that study, a small proportion of patients with high levels of TNF-a also had elevated levels of IL-6, suggesting a systemic cause for the elevation of these pro-inflammatory cytokines, which in turn may lead to a discrepancy in the assessment. Psychopathology and TNF-a. In a study using ELISA, the concentration of interleukin-6 was measured in the blood serum of 128 schizophrenia patients (24 of whom had never been treated) and in 110 healthy subjects. The mean concentration of IL-6 in the blood was significantly higher in schizophrenia patients compared with healthy subjects (P = 0.009). Comparisons within patient groups revealed that serum IL-6 was significantly associated with disease duration (p=0.0004). After variation in disease duration, there was no relationship between IL-6 levels and autoantibody production, clinical status, or medication status. Thus, elevated serum levels of IL-6 in schizophrenia develop during the course of the illness and may be related to treatment or disease progression [22], and this agrees with the results of the present study in terms of higher IL-6 concentrations in patients with schizophrenia compared to control group. The results of the current study also agreed with the study of Frommberger et al., [23], which reported an increase in the concentration of Interleukin-6 (IL-6) in patients with depression and schizophrenia, and in healthy people when stressed. Plasma levels of interleukin 6 were determined in depressed patients (n = 12) and schizophrenia patients (n = 32) during the acute disease state and after remission at approximately 8 weeks after admission and were compared with healthy controls (n = 12). Patients were diagnosed according to DSM-III-R by the Structured Clinical Interview (SLID). Illness severity was assessed for depression by the Montgomery Asberg Depression Rating Scale (MADRS) and for schizophrenia by the Brief Psychiatric Rating Scale (BPRS). An increase in interleukin 6 levels was found in plasma during the acute state of either depression or schizophrenia when compared to the control group.

Conclusions

The psychological diseases (schizophrenia, Alzheimer's) associated with stress increased the number of intestinal bacteria in feces samples of patients in comparison with healthy control group, also increased the levels of the cytokines IL-6 and TNF- α in patients compared with healthy control group.

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